

FINAL REPORT

Cover Page

BARD Project Number: IS-4469-11R

Title: Mechanisms activated by fungal-based host pH modulators during quiescent infections and active postharvest disease development

Investigator Position	Name	Affiliated Institution
1) Principal Investigator (PI)	Prusky, Dov B	ARO, Min. Ag.
2) co-PI	Mengiste, Tesfaye	Purdue
3) Collaborating	Fluhr, Robert	Weizmann Inst.

Start Date of Project: 1.12.2011

Date of Submission of Report: 30.9.2014

Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Pathogenicity, activation of quiescent infections, decay, disease control

Abbreviations commonly used in the report, in alphabetical order:

GDH2, glutamate dehydrogenases; *GSI*, glutamine synthetase; *GLT*, glutamate transporter; *MEP*, methyl ammonium permease; *AMET*, ammonium transporter; *cAMP*, cyclic AMP; *PKA*, protein kinases; *PACC*, transcription factor *pacC*.

Budget: IS: \$203.000

US: \$107.000

Total: \$310.000

____Dov Prusky____

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

- **Publication Summary (numbers)**

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	1		7	
Submitted, in review, in preparation			1	
Invited review papers				
Book chapters			1	
Books			1	
Master theses				
Ph.D. theses				
Abstracts				
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Noam Alkan and Dana Ment were supported by this grant. N.A has become a scientist in the Volcani Center at the Department of Postharvest Science, Israel

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	1	1	1	3
Longer Visits (Sabbaticals)				

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted	0	0	0	0
Issued (allowed)				
Licensed				

Description of cooperation

The cooperation was carried out at several levels:

Exchange of seeds and collaboration in experiments that were in parallel in both countries

Visit of the American and Israeli partner in Israel-and America respectively and discussion of the proper experiments in both labs.

The Aim of the project

This project aims were to provide new insights on the mechanisms activated during alkalinization and acidification of the infection court by *Colletotrichum* and *Botrytis* spp. respectively that will lead to quiescent infection-development on tomato fruits. We have chosen these pathogens due to their contrasting life style of alkalinization and acidification, respectively. We will study the roles of these fungal-based host-pH modulators in modulating host gene expression during quiescent infection development and compare these roles with those governing active colonization as a basis for developing novel strategies for control of postharvest diseases. The aims will be pursued through:

1. Characterization of the effects of pH modulation on fungal-plant cell-cell signaling and on the fungal and plant transcriptome during the initial stages of fungal quiescence. The unpublished material that is presented as short abstract is considered one of the key point modulating
2. Characterization of expression profiles of tomato fruits affected by acidifying and alkalinizing pathogens during the transformation of quiescent to active infections by *Colletotrichum* and *Botrytis*.
3. Functional analysis of selected genes involved in signaling pathways that affects the quiescent and active infections of *Colletotrichum* and *Botrytis*.

Significance of main scientific achievements or innovations.

The ability of microorganisms to sense, modulate and adapt to changes in the environment is essential for their survival (Biswas et al., 2007). This is particularly important for species with an intimate association with host organisms such as pathogens, symbionts, or commensals. One environmental factor to which microorganisms must respond is extracellular pH. In the human body, pH can vary widely, from highly acidic (pH ~2) in the stomach to mildly acidic (skin and vagina), to neutral (bloodstream and parts of the gut), and even alkaline (some parts of the gut). In fruits pH varied from 4-6 but still pathogens may increase the pH up to 7.5 or reduced it up to pH 3.2 (Prusky and Yakoby, 2003). *Candida albicans*, which is both a common fungal commensal of humans and the most important fungal pathogen of humans, thrives in most of these sites and is highly tolerant to a wide range of environmental pH conditions, from pHs of <2 to pHs of >10. For many bacterial human pathogens acquired by an oral route, the highly acidic stomach enables them to survive transient required for the earliest stages of infection in many species, (Wilmes-Riesenberg et al., 1997; Cotter et al., 1999; Merrell and Camilli, 2002; Merrell et al., 2002). Fungi are generally more acidophilic than the common pathogenic bacteria, and their response include adaptation to acidic, neutral or alkaline pH, conditions and were shown to be critical for virulence in many plant pathogens as well (Yakoby et al., 2000; Prusky et al., 2013).

C. gloeosporioides is a wide pathogen of fruits and vegetables and usually secretes massive amounts of necrotrophic non-proteinaceous effecting-molecules identified as ammonia during initial stages of penetration and necrotrophic colonization (Prusky and Yakoby, 2003; Alkan et al., 2008; Miyara et al., 2010; Prusky et al., 2010; Prusky, 1996; Wharton and Diéguez-Urbeondo, 2004). The fungus thereby increased the local pH by up to 4 pH units from pH 4.0 to pH 8.0 (Prusky et al., 2001; Prusky et al., 2013). Alkaline

adaptation has been well studied in model systems *Saccharomyces cerevisiae*, and *Aspergillus nidulans*, and *C. albicans*, (Su and Mitchell, 1993; Davis et al., 2000; Baek et al., 2006) however significant work was described related to the contribution of secreted ammonia in pathogenicity by *Colletotrichum* (Prusky et al., 2013). The accumulation of ammonia is thought to contribute to the necrotrophic colonization (Prusky and Yakoby, 2003; Prusky et al., 2013; Shnaiderman et al., 2013) by activation of host *SIRBOH* to generate reactive oxygen species and thereby accelerate local host-cell death (Alkan et al., 2009). In addition, ambient alkalization contributes to optimization of fungal pathogenicity by activation of alkaline-expressed fungal genes through a pH-sensor system mediated by PACC (Miyara et al., 2008; Alkan et al., 2013b). Other fungal pathogens secrete other necrotrophic non-proteinaceous effecting-molecules identified as organic acids that acidify the environment and contribute to optimization of fungal pathogenicity by activation of acidic-expressed fungal genes. Indeed, in many fungal species induced genes were shown to be regulated by PACC and become central pathogenicity factors including: pectate lyase (*PELB*) in *C. gloeosporioides* (Yakoby et al., 2000); polygalacturonase (*PG1* and *PG5*) in *F. oxysporum* (Caracuel et al., 2003), endoglucanases in *A. alternata* (Eshel et al., 2002) and polygalacturonase in *P. expansum* (Prusky et al., 2004). Irrespective of their optimal pH preference, fungi detect pH with the PAL-PACC signal transduction system (Espeso and Arst, 2000). In *C. gloeosporioides*, under alkaline pH conditions, PACC serves as a positive regulator, promoting transcription of alkaline-expressed genes (Caracuel et al., 2003; Alkan et al., 2013b) and it was hypothesized to simultaneously repress acid-expressed genes by a new described mechanism involving AREB activation (Caracuel et al., 2003; Ment et al., 2014). These mechanisms of pH modulation of pathogenicity factors is thought to orchestrate optimal combinations of gene expression (Rollins, 2003).

The assessment of the PACC regulated genes indicate the presence of 478 PACC up-regulated genes transcripts that were characterized as alkaline-expressed, whereas 483 down-

regulated genes that were characterized as acid-expressed (Alkan et al., 2013b). Interestingly, different members of the same gene families (transporters, antioxidants and cell-wall-degrading enzymes) were found to belong to either PACC up- (alkaline-expressed) or down-regulated (acid-expressed) groups suggesting that similar type of gene activities are expressed in both pH conditions (Ment et al., 2014). The differential, pH-dependent expression of genes with similar activities suggested that genes are selectively activated on the basis of their optimal enzymatic pH activity, thus allowing the fungus to cope with variable pH conditions and to make optimal use of its inventory of available enzymes. This is important since the mechanism of activation of PACC is not to switch a new gene on or off, but rather to assist in the continuous fine-tuning of the expression of transporters, antioxidants and CWDEs, so as to maintain homeostasis and expression of pathogenicity factors for orchestration of the genomic arsenal under changing pH (Alkan et al., 2013b).

A much less well understood aspect of pH adaptation and regulation is the ability to actively alter extracellular pH. This is strong importance since fungal pathogens as *Colletotrichum* may alkalize and *Penicillium* may acidify as a factor modulating pathogenicity species (Yakoby et al., 2000; Prusky et al., 2013; Shnaiderman et al., 2013). But while in plant pathogen the pH modulation is activated as an improved mechanism of pathogenicity in humans pathogens as *Helicobacter pylori* pH adaptation is used to tolerate the highly acidic pH of the stomach by expressing a urease, found in part on the cell surface, that produces ammonia and creates an alkaline microenvironments with more hospitable pH (Eaton et al., 1991; Tsuda et al., 1994). Also dermatophytic fungi are identified based on a slow alkalization phenomenon assayed over 10 to 14 days (Taplin et al., 1969). There are several fungal species as *Neurospora crassa*, *A. fumigatus*, *Metarhizium anisopliae*, and *S. cerevisiae* that have a significant ability to respond to pH, but interestingly have limited ability to alkalinize the extracellular milieu (St. Leger et al., 1999; Palková et al., 1997). In contrast, *C. albicans* and *C. gloeosporioides* have a remarkable ability to alter extracellular pH, by the

secretion of ammonia, creating a neutral/alkaline environment from either acidic or alkaline starting conditions, with changes of >3 pH units (Vylkova et al. 2011; Prusky, D., and Yakoby, N. 2003). Our work was concentrated in the pH modulation of host fruit as a mechanism for fungal attack. Plant pathogens, and specifically postharvest pathogens remain quiescent during fruit growth following the infection of the fruit and during fruit ripening they modulate the host pH to enhance the colonization of ripe fruit, (Prusky et al., 2013). During fruit ripening the fruits significantly change their content by transforming complex sugar to more soluble ones (Prusky, 1996). This may result in increased concentration of available sugars that may reach up to 14-20% total soluble solids (Hulme, 1971). Little is known on the importance of host nutritional level on the pH modulating life pattern induced by the secretable effecting molecules. In the future the importance of nutritional factors in modulating pathogenicity should be studied.

Agricultural and/or economic impacts of the research findings, if known.

Our objectives in previous studies were to determine the influence of external acid treatment on the pathogen and on its symptom development. We also analyzed the effects of acidic solutions on the solubility of prochloraz fungicide, and on its biological activity in the control of alternaria rot in subtropical fruit. Our findings indicate that acid treatment may, in itself, reduce symptom development by *A. alternata*, and it may improve the efficiency of disease control by prochloraz. Acid treatment represents a new approach that is widely used for the control of postharvest diseases of mango fruits In Israel. The acid treatment contributed to disease control and fruit-quality improvement; at the same time it improves safety by reducing the concentration of prochloraz used as a postharvest fungicide application. Prusky, D., Kobiler, I., Akerman, M., and Miyara, I. 2006. Effect of acidic solutions and acidic prochloraz on the control of postharvest decay caused by *Alternaria alternata* in mango and persimmon fruits. *Postharvest Biology and Technology* 42: 134-141

Evaluation of research achievements

I. Characterization of the effect of pH modulation on the fungus

1. Role of Nitrogen-Metabolism Genes Expressed During Pathogenicity of the Alkalinizing *Colletotrichum gloeosporioides* and Their Differential Expression in Acidifying Pathogens

I. Miyara, C. Shnaiderman, X. Meng, W. A. Vargas, J. M. Diaz-Minguez, A. Sherman, M. Thon, and **D. Prusky**. 2012. Role of Nitrogen-Metabolism Genes Expressed during Pathogenicity of the Alkalinizing *Colletotrichum gloeosporioides* and their Differential Expression in Acidifying Pathogens. *Molecular Plant Microbe Interaction* 25:1251-63.

Pathogens can actively alter fruit pH around the infection site, signaling modulation of pathogenicity-factor expression, as found for alkalinizing (*Colletotrichum* and *Alternaria* spp.) and acidifying (*Penicillium*, *Botrytis*, and *Sclerotinia* spp.) fungi. The nitrogen-metabolism genes *GDH2*, *GS1*, *GLT*, and *MEP* genes are differentially expressed during colonization by *Colletotrichum gloeosporioides*, and a $\Delta gdh2$ strain reduces ammonia accumulation and pathogenicity. We analyzed the contribution of transporters *GLT* and *MEPB* to *C. gloeosporioides* pathogenicity. Germinating spores of Δglt strains showed reduced appressorium formation; those of $\Delta mepB$ mutants showed rapid ammonia uptake and accumulation inside the hyphae, indicating deregulated uptake. Both mutants reduced pathogenicity, indicating that these transporters function during alkalinizing species pathogenicity. We compared the expressions of these genes in *C. gloeosporioides* and *Sclerotinia sclerotiorum*, and found five to 10-fold higher expression at the transcript level in the former. Interestingly, *GLT* and *MEPB* in the alkalinizing species showed no and very low sequence identity, respectively, with their counterparts in the acidifying species. Knockout analysis of *GLT* and *MEPB* and their differential transcript regulation in the alkalinizing and acidifying species suggest that the ammonia accumulation contributing to pathogenicity in the former is modulated by factors at the gene-regulation levels that are lacking in the acidifying species.

2. Differential activation of ammonium transporters during the accumulation of ammonia by *Colletotrichum gloeosporioides* and its effect on appressoria formation and pathogenicity

Chen Shnaiderman, Itay Miyara, Ilana Kobiler, Amir Sherman, and **Dov Prusky**. 2013. Differential activation of ammonium transporters during the accumulation of ammonia by *Colletotrichum gloeosporioides* and its effect on appressoria formation and pathogenicity. *Molecular Plant Microbe Interaction*: 26:345-55.

Ammonium secreted by the post-harvest pathogen *Colletotrichum gloeosporioides* during host colonization accumulates in the host environment due to enhanced fungal nitrogen metabolism. Two types of ammonium transporter encoding genes, *AMET* and *MEP*, are expressed during pathogenicity. Gene disruption of *AMET*, a gene modulating ammonia secretion, showed twofold reduced ammonia secretion and 45% less colonization on avocado fruit, suggesting a contribution to pathogenicity. *MEPB*, a gene modulating ammonium transport, is expressed by *C. gloeosporioides* during pathogenicity and starvation conditions in culture. Gene disruption of *MEPB*, the most highly expressed gene of the *MEP* family, resulted in twofold overexpression of *MEPA* and *MEPC* but

reduced colonization, suggesting *MEPB* expression's contribution to pathogenicity. Analysis of internal and external ammonia accumulation by $\Delta mepB$ strains in mycelia and germinated spores showed rapid uptake and accumulation, and reduced secretion of ammonia in the mutant versus wild-type (WT) strains. Ammonia uptake by the WT germinating spores but not by the $\Delta mepB$ strain with compromised ammonium transport activated cAMP and transcription of *PKA* subunits *PKAR* and *PKA2*. $\Delta mepB$ mutants showed 75% less appressorium formation and colonization than the WT, which was partially restored by 10 mM exogenous ammonia. Thus, whereas both *AMET* and *MEPB* genes modulate ammonia secretion, only *MEPB* contributes to ammonia accumulation by mycelia and germinating spores that activate the cAMP pathways, inducing the morphogenetic processes contributing to *C. gloeosporioides* pathogenicity

3. Global aspects of *pacC* regulation of pathogenicity genes in *Colletotrichum gloeosporioides* revealed by transcriptome analysis

Noam Alkan, Xiangchun Meng, Gilgi Friedlander, Eli Reuveni, Serenella Sukno, Amir Sherman, Michael Thon, Robert Fluhr, and Dov Prusky. Genomic and transcriptomic analysis of *Colletotrichum gloeosporioides* reveals a conserved role for *pacC* pH regulation in Fungi. *Molecular Plant-Microbe Interactions*. 26: 1345–1358

Colletotrichum gloeosporioides alkalinizes its surroundings during colonization of host tissue. The transcription factor *pacC* is a regulator of pH-controlled genes and is essential for successful colonization. We present here the sequence assembly of the *Colletotrichum* fruit pathogen and use it to explore the global regulation of pathogenicity by ambient pH. The assembled genome size was 54 Mb, encoding 18,456 genes. Transcriptomes of the wild type and $\Delta pacC$ mutant were established by RNA-seq and explored for their global pH-dependent gene regulation. The analysis showed that *pacC* upregulates 478 genes and downregulates 483 genes, comprising 5% of the fungal genome, including transporters, antioxidants, and cell-wall-degrading enzymes. Interestingly, gene families with similar functionality are both up- and downregulated by *pacC*. Global analysis of secreted genes showed significant *pacC* activation of degradative enzymes at alkaline pH and during fruit infection. Select genes from alkalizing-type pathogen *C. gloeosporioides* and from acidifying-type pathogen *Sclerotinia sclerotiorum* were verified by quantitative reverse transcription polymerase chain reaction analysis at different pH values. Knock out of several *pacC*-activated genes confirmed their involvement in pathogenic colonization of alkalized surroundings. The results suggest a global regulation by *pacC* of key pathogenicity genes during pH change in alkalizing and acidifying pathogens.

4. Virulence Regulation of Phytopathogenic Fungi by pH

Alkan, N., Espeso, E. A., and Prusky, D. 2013. Virulence Regulation of Phytopathogenic Fungi by pH. *Antioxidant and Redox Signaling*: 19:1012-25. doi: 10.1089/ars.2012.5062.

Postharvest pathogens can start its attack process immediately after spores land on wounded tissue, whereas other pathogens can forcibly breach the unripe fruit cuticle and then remain quiescent for months until fruit ripens and then cause major losses.

Recent Advances: Postharvest fungal pathogens activate their development by secreting organic acids or ammonia that acidify or alkalinize the host ambient surroundings. Critical Issues: These fungal pH modulations of host environment regulate an arsenal of enzymes to increase fungal pathogenicity. This arsenal includes genes and processes that

compromise host defenses, contribute to intracellular signaling, produce cell wall-degrading enzymes, regulate specific transporters, induce redox protectant systems, and generate factors needed by the pathogen to effectively cope with the hostile environment found within the host. Further, evidence is accumulating that the secreted molecules (organic acids and ammonia) are multifunctional and together with effect of the ambient pH, they activate virulence factors and simultaneously hijack the plant defense response and induce program cell death to further enhance their necrotrophic attack.

Future Directions: Global studies of the effect of secreted molecules on fruit pathogen interaction, will determine the importance of these molecules on quiescence release and the initiation of fungal colonization leading to fruit and vegetable losses. Antioxid. Redox Signal. 00, 000–000.

5. Lifestyle transitions in plant pathogenic *Colletotrichum* fungi deciphered by genome and transcriptome analyses

Lifestyle transitions in plant pathogenic *Colletotrichum* fungi deciphered by genome and transcriptome analyses. 2014. O’Connell R., Thon M R, Hacquard S, van Themaat E V L, Amyotte S G, Kleemann J, Torres M F, Damm U., Buiate E A, Epstein L, Alkan N, Altmüller J, Alvarado-Balderrama L, Bauser C L, Becker C, Birren B W, Chen Z, Choi J, Crouch J A, Duwick J P, Farman M L, Gan P, Heiman D, Henrissat B, Howard R J, Kabbage M, Koch C, Kubo Y, Law A D, Lebrun M H, Lee Y H, Miyara I, Moore N, Neumann U, Panaccione D G, Panstruga R, Place M, Proctor R H, **Prusky D**, Rech G, Reinhardt R, Rollins J A, Rounsley S, Schard C L, Schwartz D C, Shenoy N, Shirasu K, Sikhakolli U R, Stüber K, Sukno S A, Sweigard J A, Takano Y, Takahara H, Trail F, van der Does H C, Voll L M, Will I, Young S, Zeng Q, Zhang J, Zhou S, Dickman M B, Schulze-Lefert P, Ma L J, Vaillancourt L J. 2012. Life-style transitions in plant pathogenic *Colletotrichum* fungi deciphered by genome and transcriptome analyses. Nature Genetics 44:1060–1065.doi:10.1038/ng.2372.

http://www.nature.com/ng/journal/v44/n9/full/ng.2372.html?WT.ec_id=NG-201209.

Colletotrichum species are fungal pathogens that devastate crop plants worldwide. Host infection involves the differentiation of specialized cell types that are associated with penetration, growth inside living host cells (biotrophy) and tissue destruction (necrotrophy). We report here genome and transcriptome analyses of *Colletotrichum higginsianum* infecting *Arabidopsis thaliana* and *Colletotrichum graminicola* infecting maize. Comparative genomics showed that both fungi have large sets of pathogenicity-related genes, but families of genes encoding secreted effectors, pectin-degrading enzymes, secondary metabolism enzymes, transporters and peptidases are expanded in *C. higginsianum*. Genome-wide expression profiling revealed that these genes are transcribed in successive waves that are linked to pathogenic transitions: effectors and secondary metabolism enzymes are induced before penetration and during biotrophy, whereas most hydrolases and transporters are upregulated later, at the switch to necrotrophy. Our findings show that preinvasion perception of plant-derived signals substantially reprograms fungal gene expression and indicate previously unknown functions for particular fungal cell types.

6. The Regulation of the Transcription factors *pacC* and *areB* on the Acid-Expressed Genes in *Colletotrichum gloeosporioides* and their effect on Pathogenicity

The Regulation of the Transcription factors *pacC* and *areB* on the Acid-Expressed Genes in *Colletotrichum gloeosporioides* and their effect on Pathogenicity. 2014. Dana Ment,

Noam Alkan, Neta Luria, Fang-Cheng Bi, Eli Reuveni, Robert Fluhr, and **Dov Prusky**, 2014: Molecular Plant Microbe Interactions: In press

Gene expression regulation by pH in filamentous fungi and yeasts is controlled by PACC/RIM101 transcription factor. In *Colletotrichum gloeosporioides* *pacC* is known to act as positive regulator of alkaline-expressed genes, which contribute to fungal pathogenicity. However PACC is also a negative regulator of acid expressed genes, but the mechanism of down-regulation of acid-expressed genes by PACC and their contribution to *C. gloeosporioides* pathogenicity is not well understood. RNA sequencing was employed to analyze the global expression of pH-controlled genes in *C. gloeosporioides* wild-type and *pacC*-deletion mutant. The analysis demonstrated that PACC transcription factor binding sites (TFBSs) are significantly over-represented in the promoter of *pacC*-up-regulated, alkaline-expressed genes. In contrast, they are not over-represented in *pacC*-down-regulated, acid-expressed genes; instead, acid-expressed genes showed over-representation of *areB* GATA TFBS in *C. gloeosporioides* and in homologs of five other ascomycetes genomes. *areB* promoter contains *pacC* TFBS, its transcript was up-regulated at pH 7 and repressed in $\Delta pacC$. Furthermore, acid-expressed genes were found to be constitutively up-regulated in $\Delta areB$ during alkalizing conditions. *areB* mutants showed significantly reduced ammonia secretion and pathogenicity on tomato fruits. Present results indicate that *pacC* activates *areB*, thereby conditionally repressing acid-expressed genes, and contributing critically to *C. gloeosporioides* pathogenicity.

7. Carbon regulation of environmental pH by non-protein secreted effectors modulated pathogenicity by *C. gloeosporioides* and other fungi

Carbon regulation of environmental pH by non-protein secreted effectors modulated pathogenicity by *C. gloeosporioides* and other fungi. Fangcheng Bi, Shiri Barad, Dana Ment, Virginia Casado, Neta Luria, Jose Diaz Mínguez, Robert Fluhr and Dov Prusky: Unpublished material.

Nutritional factors modulate pH environment and life pattern of pathogenic fungi during host colonization leading to the specific induction of gene modulation that contributes to necrotrophic development. We have presently shown that several fungal species pathogens as *Colletotrichum*, *Penicillium*, *Sclerotinia* and *Fusarium* secrete non-proteinaceous effecting molecules that differentially modulate the culture media and host environment pH, resulting in the induction of either acid or alkaline life patterns inducing the PACC dependent activation of genes by according to the specific colonization conditions.. In the present work we have demonstrated that the secretion of effecting-molecules that modulate the acidic or alkaline life patterns and contribute to fungal pathogenicity, are carbon dependent and probably regulated by the negatively-acting zinc finger repressor *CREA*. Acidification is induced under sucrose excess 175 mM sucrose, and *creA* up regulation. This preferred carbon sources is catalyzed by the oxidation of glucose to gluconic acid (GLA) by *gox2*, while alkalization occurs under carbon deprivation, 15 mM sucrose, and *creA* down regulation, as a result of catalyzed deamination of non-preferred carbon sources as the amino acid glutamate by *gdh2* leading to ammonia secretion. Developing functional mutants of $\Delta gdh2$ and $\Delta gox2$ and differential pathogenicity in host containing different sugar levels were used to support the importance of carbon regulation of process inducing pathogenicity in several fungal species. While colonization of *C. gloeosporioides* on avocado and tomato fruits with relative low sugar level of 5-6% induced ammonia accumulation and pH increase, fruits with high sugar content, 14%, induced GLA accumulation and pH decrease by the same pathogen. Present results indicate that pH modulation is a wide mechanism between

fungus species that modulated the pH environment of plant pathogens and their regulation may have significant contribution plant health. Present results indicate the wide importance of nutritional availability for pathogens to survive in environments with limited and changing resources leading to differential pH modulation and the consequent stimulation of PACC –dependent genes and its contribution to pathogenesis.

II. Characterization of fungal and tomato fruit transcriptome

1. Quiescent and Necrotrophic Lifestyle Choice During Postharvest Disease Development

Quiescent and Necrotrophic Lifestyle Choice During Postharvest Disease Development. 2013. **Dov Prusky**, Noam Alkan, Tesfaye Mengiste, and Robert Fluhr. *Annu. Rev Phytopathol.* 51:155-76.

Insidious fungal infections by postharvest pathogens remain quiescent during fruit growth until, at a particular phase during fruit ripening and senescence, the pathogens switch to the necrotrophic lifestyle and cause decay. During ripening, fruits undergo physiological processes, such as activation of ethylene biosynthesis, cuticular changes, and cell wall loosening—changes that are accompanied by a decline of antifungal compounds, both those that are preformed and those that are inducible secondary metabolites. Pathogen infection of the unripe host fruit initiates defensive signal-transduction cascades, culminating in accumulation of antifungal proteins that limit fungal growth and development. In contrast, development of the same pathogens during fruit ripening and storage activates a substantially different signaling network, one that facilitates aggressive fungal colonization. This review focuses responses induced by the quiescent pathogens of postharvest diseases in unripe host fruits. New genome-scale experimental approaches have begun to delineate the complex and multiple networks of host and pathogen responses activated to maintain or to facilitate the transition from the quiescent to the necrotrophic lifestyle.

2. Simultaneous transcriptome analysis of *Colletotrichum gloeosporioides* and tomato fruits response reveals novel fungal-fruit arm and defense strategies

Alkan N, Friedlander G, Ment D, **Prusky D** and R. Fluhr. 2014. Simultaneous transcriptome analysis of *Colletotrichum gloeosporioides* and tomato fruits response reveals novel fungal-fruit arm and defense strategies. *New Phytologist*: In press.

The fungus, *Colletotrichum gloeosporioides*, breaches the fruit cuticle but remains quiescent until fruit ripening signals a switch to necrotrophic culminating in economically devastating anthracnose disease. There is a need to understand the distinct fungus life-style and the simultaneous fruit response.

Fungal-fruit interactions transcriptome analysis was carried out in the appressoria, quiescent and necrotrophic stages. Fungal appressoria development on unripe fruit showed stage-specific transcription and was accompanied by massive fruit transcriptional defense response. The quiescent stage showed discrete fungal morphology including; dendritic like structures in the fruit cuticle and swollen hyphae in the first epidermal cell layer. The quiescent fungal transcriptome showed activation of chromatin remodeling genes while the fruit response continued by highly integrated massive up-regulation of defense genes. During infection of ripe fruit, fungi recapitulate the same developmental stages in a shorter time sequence. The necrotrophic stage showed a dramatic shift in up-regulation of

C. gloeosporioides pathogenicity factors and a susceptible fruit response that shows activation of the salicylic acid pathway culminating in cell death and anthracnose disease. Transcriptome analysis of *C. gloeosporioides* infection of fruit reveals its distinct stage-specific life style and the concurrent fruit response, deepening our perception of the unfolding fungal-fruit arms and defenses race.

Invited Reviews

Prusky, D., and Kobiler, I. 2012. Mechanism modulating the activation of quiescent infections by postharvest pathogens. In: Avancos Tecnologicos na Patologia Pos-Colheita. Ed. Sonia Maria Alves de Oliveira, Severina Rodrigues de Oliveira Lis and Alice Maria Goncalves Santos, org.-- EDUFRPE, 2012.

Prusky, D., Barad, S., Luria, N. Ment, D. 2014. pH Modulation of Host Environment, a Mechanism Modulating Fungal Attack in Postharvest Pathogen Interactions. In D. Prusky, M.L. Gullino (eds.), Postharvest Pathology, Plant Pathology in the 21st Century 7, DOI 10.1007/978-3-319-07701-7_2

Changes in direction, if any, from that in the original proposal.

No change in direction from that of the original proposal was obtained.

List of publications arising from the joint research.

- Alkan, N., Fluhr, R., and Prusky, D. 2012. *Colletotrichum coccodes* infection of ripe and unripe tomato fruit is modulated by ammonium secretion through salicylic and jasmonic acid pathways leading to PCD and differential colonization. **Molecular Plant Microbe Interaction**: 25:85-96.
- O'Connell R., Thon M R, Hacquard S, van Themaat E V L, Amyotte S G, Kleemann J, Torres M F, Damm U., Buiate E A, Epstein L, Alkan N, Altmüller J, Alvarado-Balderrama L, Bauser C L, Becker C, Birren B W, Chen Z, Choi J, Crouch J A, Duvick J P, Farman M L, Gan P, Heiman D, Henrissat B, Howard R J, Kabbage M, Koch C, Kubo Y, Law A D, Lebrun M H, Lee Y H, Miyara I, Moore N, Neumann U, Panaccione D G, Panstruga R, Place M, Proctor R H, Prusky D, Rech G, Reinhardt R, Rollins J A, Rounsley S, Schard C L, Schwartz D C, Shenoy N, Shirasu K, Sikhakolli U R, Stüber K, Sukno S A, Sweigard J A, Takano Y, Takahara H, Trail F, van der Does H C, Voll L M, Will I, Young S, Zeng Q, Zhang J, Zhou S, Dickman M B, Schulze-Lefert P, Ma L J, Vaillancourt L J. 2012. Life-style transitions in plant pathogenic *Colletotrichum* fungi deciphered by genome and transcriptome analyses. **Nature Genetics** 44:, 1060–1065. doi:10.1038/ng.2372.
- Miyara, I., Shnaiderman, C., Meng, X., Vargas, W. A., Diaz-Minguez, J. M., Sherman, A., Thon, M., and Prusky, D. 2012. Role of Nitrogen-Metabolism Genes Expressed during Pathogenicity of the Alkalinizing *Colletotrichum gloeosporioides* and their Differential Expression in Acidifying Pathogens. **Molecular Plant Microbe Interaction** 25:1251-63.
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